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published in

Aquatic Ecosystem Health and Management
2000

DOI (link to publisher)

[10.1016/s1463-4988\(00\)00035-x](https://doi.org/10.1016/s1463-4988(00)00035-x)

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

den Besten, P. J., Postma, J., Wegener, J. W. M., Keidel, H., Klink, A., Mol, J., & van de Guchte, C. (2000). Biological and chemical monitoring after pilot remediations in the delta of the rivers Rhine and Meuse. *Aquatic Ecosystem Health and Management*, 3, 317-334. [https://doi.org/10.1016/s1463-4988\(00\)00035-x](https://doi.org/10.1016/s1463-4988(00)00035-x)

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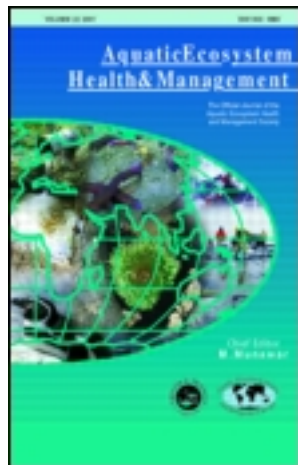
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Aquatic Ecosystem Health & Management

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/uaem20>

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Version of record first published: 07 Nov 2008.

To cite this article: P.J. den Besten, J.F. Postma, J.W.M. Wegener, H. Keidel, A. Klink, J. Mol & C. van de Guchte (2000): Biological and chemical monitoring after pilot remediations in the delta of the rivers Rhine and Meuse, *Aquatic Ecosystem Health & Management*, 3:3, 317-334

To link to this article: <http://dx.doi.org/10.1080/14634980008657030>

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Biological and chemical monitoring after pilot remediations in the delta of the rivers Rhine and Meuse[☆]

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Abstract

Two pilot projects were carried out to investigate the effects of sediment remediation. One project was situated in a groyne section of the Nieuwe Merwede, a watercourse in the Rhine delta. The second was situated in a creek named Spijkerboor, which receives water from the river Meuse. For both sites it was concluded earlier that sediment pollution posed a high risk to the ecosystem. The remediations consisted of partial excavation of the contaminated sediments, followed by application of a clean layer of sandy material on top of the remaining contaminated sediment. Before and at various times after the remediation, the following investigations were carried out: physical–chemical analyses of sediment, benthic community structure observations, bioaccumulation measurements and sediment bioassays. After the remediation, a new silty sediment top layer was formed with lower contaminant levels exhibiting a lower toxicity. In the remediated site in the Nieuwe Merwede, chironomids, oligochaetes and nematodes reappeared within 3–7 months at normal densities, while in the Spijkerboor recolonization by chironomids and nematodes proceeded more slowly. After 2 years, in both the remediated sites chironomids and nematodes were present in densities much higher than before the remediation. Bivalves showed a low recolonization rate in both sites. In non-remediated, polluted reference sites also a lower sediment toxicity and a nearly comparable recovery of the benthic community was observed, probably the result of natural sedimentation of material with lower contaminant levels. Because of the natural improvement of sediment quality, the net effects of remediation were negligible in the Spijkerboor. For the Nieuwe Merwede, after 2 years still lower contaminant levels were observed in the remediated site compared to the non-remediated site, resulting in lower bioaccumulation in oligochaetes. However, the contaminant levels in sediment and biota still do not meet all

[☆] Paper presented at the 3rd International Symposium on Sediment Quality Assessment—Ecological Hazard & Risk Assessment in Aquatic Environment: Science and Strategies in Remediation, Restoration and Rehabilitation, Amsterdam 18–19 August 1998. Sponsored by the Aquatic Ecosystem Health & Management Society/AEHMS; Institute of Inland Water Management and Waste Water Treatment/RIZA; Netherlands Expert Centre on Contaminated Sediments/AKWA and Netherlands Society for Toxicology, Section Environmental Toxicology/NVT.

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environmental quality objectives. Based on the outcome of this study it is recommended that the priority for remedial action is made dependent on the rate of natural covering with sediments from the rivers Rhine and Meuse. © 2000 Elsevier Science Ltd and AEHMS. All rights reserved.

Keywords: Sediment contamination; Benthic community; Meiofauna; Macrofauna; Colonization; Bioaccumulation; Sediment bioassays

1. Introduction

In the 1970s, millions of cubic metres of highly polluted sediments were deposited in the delta of the rivers Rhine and Meuse. Heavily polluted, silt-rich sediments are found especially in the groyne sections of the rivers Rhine and Meuse, in the creeks of the floodplain forests of the Biesbosch (an important nature-reserve area) and, further downstream in the delta, in the deeper parts of the Hollandsch Diep and the Haringvliet. In 1992 research was started to evaluate the risks of sediment contamination for the ecosystem. Attention was given not only to possible direct effects on the benthic community, but also to indirect effects that can occur as a result of the accumulation of contaminants in foodchains (Den Besten et al., 1995).

During the last 15 years contaminant levels and toxicity of suspended solids in the water of the rivers Rhine and Meuse dropped considerably (Admiraal et al., 1993; Hendriks et al., 1994). Therefore, the question was raised whether the delta would benefit from remedial actions.

From autumn 1995 to spring 1996, two pilot remediation projects were carried out simultaneously to investigate whether environmental risks can be reduced by dredging polluted sediment. One project was situated in a groyne section of the Nieuwe Merwede, a watercourse in the Rhine delta (Fig. 1). The second was situated in a large creek in the Brabantsche Biesbosch. This creek, named Spijkerboor, receives water from the river Meuse (Fig. 1). The remediations consisted of partial excavation of the contaminated sediments, followed by application of a clean layer of sandy material on top of the remaining contaminated sediment (for a detailed description, see Van Meel et al., 1997). An extensive monitoring programme accompanied both projects. The aim of the investigations was to evaluate the recovery of the meio- and macrozoobenthos

communities, and to compare pollution-related effects before and after the remediation. The latter was done on the basis of physical-chemical analyses of sediment, sediment bioassays and bioaccumulation measurements. The present paper describes the results of the biological and chemical-monitoring programme in the period 1992–1998.

2. Methods and materials

The geographical position of the two pilot remediation sites is shown in Fig. 1. Upstream of both pilot remediation sites, one or more reference sites were chosen to follow sediment quality development in the non-remediated situation. In each site a number of different sampling points were chosen (Fig. 1). At each sampling point sediment was collected for chemical analyses, bioassays and a survey of the macro- and meiofauna. Sampling was carried out in 1992, 1993, 1995, 1997 and 1998, in early spring (March and April) each year, because the aim of the survey was to characterize the macro- and meiozoobenthos community that had survived the previous winter. Additionally, directly after the pilot remediations (spring 1996), samples for nematode analysis were taken in June, August and October 1996; sampling was also carried out for a macrofauna survey and bioassays in October 1996. The sediment for bioaccumulation bioassays was collected in September 1997.

2.1. Collection of sediment samples

The sediment samples for chemical analyses, macrozoobenthos surveys and bioassays were collected with a $0.2 \times 0.3 \text{ m}^2$ box-corer or with small corers. For macrozoobenthos studies three box-corer grabs (6 l of top layer sediment each) were taken at each sampling point. At the shallowest sampling points, 18 grabs were taken with a 65-mm diameter corer and pooled to give the same sampling area (this procedure in triplicate). The sediment was

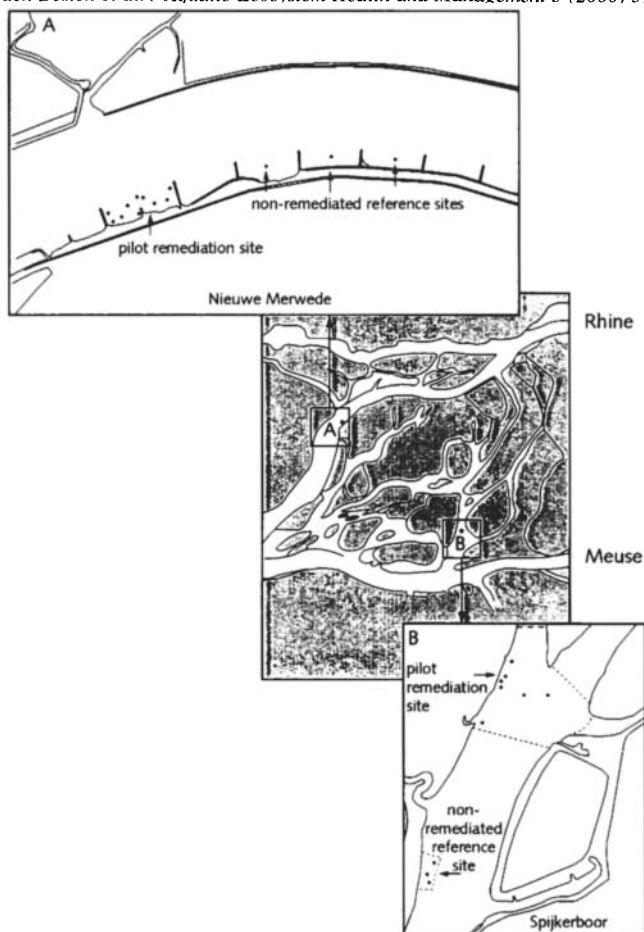


Fig. 1. Map of the pilot remediation site in the Nieuwe Merwede (a water course in the Rhine delta) and the pilot remediation site in the Spijkerboor (part of the former floodplain forest Brabantse Biesbosch, receiving water from the river Meuse). The area of the remediation site in the Nieuwe Merwede is approximately 40,000 m², that of the remediation site in the Spijkerboor is approximately 96,000 m². Dots represent sampling points of the monitoring after the remediations.

sieved immediately on a 500- μ m mesh sieve after which the macrofauna was conserved in 6% formaldehyde. For the analysis of the nematode community, a 44-mm diameter corer was used to sample the top layer material from the sediment in a box-corer grab. In shallow sites, cores were taken directly from the sediment. Eight cores (1.2 l) were transferred to a glass bottle, after which the wet sediment was mixed gently with 60 ml of 37% formaldehyde and stored at 4°C. For the bioassays the 10-cm top layer of several cores was pooled and stored in glass bottles or PVC buckets at 4°C until use (maximum storage time: 1 month).

2.2. Chemical analysis (sediment)

The chemical analyses were carried out as described in Den Besten et al. (1995) and Reinhold-Dudok van Heel and Den Besten (1999). The contaminant levels were normalized according to the approach described by CUWVO (1990) in order to compensate for differences in the sorption characteristics between sediments; standard sediment was defined as having a 25% particle fraction <2 μ m and 10% organic matter on a dry weight basis. The normalized contaminant levels were compared with the Dutch sediment quality criteria. The final

sediment classification ranges from class 0 (not contaminated) to class 4 (highly contaminated; for class 4 sediments remediation can be enforced by law) based on the highest classification of individual contaminants (Ministry of Transport, Public Works and Water Management, 1994).

2.3. Survey of macrozoobentos community and nematode population

Macrofauna species were identified according to standard literature and nomenclature (see Den Besten, 1997). Results were presented as abundances (number of animals m^{-2}), total biomass (calculated from biovolume measurements, see Smit and Dudok van Heel, 1992; Smit et al., 1993), and/or the number of species within different taxonomic groups (chironomids, bivalves, oligochaetes, and the group of the more rare species of Trichoptera, Ephemeroptera and Plecoptera). For chironomids the incidence of mentum deformities (Warwick, 1988) of *Chironomus* species and the ratio between the abundance of *Chironomus* larvae and the total of *Chironomus* and *Procladius* species (CCP index; adapted from Warwick, 1991) were used as additional parameters. As overall diversity indices, the Shannon–Wiener index and the Simpson index were calculated (Shannon and Weaver, 1949; Simpson, 1949). Three replicates were analysed per sampling point; results for variable numbers of sampling points were averaged to site means.

Nematodes were extracted from the sediment according to the method described by Bongers and Van de Haar (1990). Up to 100 specimens were identified to species level according to descriptions given in standard literature. Results were presented as the total abundance (number of animals m^{-2}), the number of species and the maturity index (Bongers, 1990).

2.4. Effect bioassays

Sediment toxicity was assessed using bioassays with the following species:

- *Chironomus riparius* (whole sediment bioassay with midge larvae);
- *Daphnia magna* (sediment pore water bioassay with water fleas);
- *Vibrio fischeri* (Microtox assay with sediment pore water/elutriate);
- *Thamnocephalus platyurus* (sediment pore water bioassay with a freshwater crustacean);
- *Brachionus calyciflorus* (sediment pore water bioassay with rotifers).

The bioassay with *Chironomus riparius* (duration 28 days) was performed according to the draft OECD guideline for the testing of chemicals (Van de Guchte et al., 1993; Den Besten et al., 1995). The following end points were used: % hatched eggs, mortality during development, % larvae with retarded development and the mean dry weight of fourth instar larvae. For each end point the significance of effects was tested by analysis of variance (ANOVA), comparing the mean of four test sediment replicates with the mean of the reference sediment (sand or silt, depending on the sediment type of the tested sediments). In case criteria for performing ANOVA were not met (even after transformation) the non-parametric Kruskal–Wallis test was used instead (Sokal and Rohlf, 1981). The results were classified as follows:

- : no effect, >75% hatched larvae, mortality/retardation in development $\leq 10\%$, and effect on dry weight $\leq 10\%$;
- ± : moderate effect, 50–75% hatched larvae, or mortality/retardation in development 10–50%, or effect on dry weight 10–25%;
- + : strong effect, less than 50% hatched larvae, or mortality/retardation in development $\geq 50\%$, or effect on dry weight $\geq 25\%$.

Only significant differences between test sediments and reference sediments were taken into account; the resulting classification was based on the most sensitive parameter.

Bioassays with *D. magna* were carried out in sediment pore water according to standard methods for sediment toxicity tests as described by Maas et al. (1993) and Den Besten et al. (1995). Mortality and reproduction were used as end points. Tests were performed until three breed releases had been observed in the blank (after 14–16 days). The no observed effect concentration ($NOEC_{mortality}$) was defined as the highest concentration of pore water

(in % v/v) in which mortality was $\leq 20\%$. Reproduction was evaluated by calculating the intrinsic rate of population growth (r_m ; Van Leeuwen et al., 1985) and testing the difference (in case of a decrease) with the r_m in the corresponding blank statistically according to Williams (1971, 1972). The $\text{NOEC}_{\text{reproduction}}$ was defined as the highest concentration of pore water in which the r_m was not significantly different from that found for the blank. The results were classified as follows:

- : no effect, $\text{NOEC}_{\text{mortality/reproduction}} = 100\%$ pore water;
- \pm : moderate effect, $10\% < \text{NOEC}_{\text{mortality/reproduction}} < 100\%$ pore water;
- + : strong effect, $\text{NOEC}_{\text{mortality/reproduction}} \leq 10\%$ pore water, or mortality in 100% pore water within 48 h $\geq 50\%$.

The resulting classification was based on the most sensitive parameter.

The bioassay with *V. fischeri* was performed with samples of pore water (see above; for this bioassay no filtration after centrifugation) according to Dutch Standard Methods (NVN 6516, 1993). The samples were tested in duplicate using a range of four pore water dilutions (6–45% v/v), by measuring the decrease in fluorescence after 5, 15 and 30 min. The results were based on the time at which the largest effect was observed. The concentration of pore water, which gave 20% inhibition of fluorescence (EC_{20}), was calculated after correction for changes in fluorescence occurring in blanks which were tested simultaneously. Finally the toxicity index (TI) was calculated: $\text{TI} = 1/\text{EC}_{20} \times 100$. The results were classified as follows:

- : no effect, $\text{TI} < 2$;
- \pm : moderate effect, $2 \leq \text{TI} < 10$;
- + : strong effect, $\text{TI} \geq 10$.

The bioassay with the rotifer *B. calyciflorus* (Rotoxkit™) was performed with sediment pore water (preparation as for bioassays with *D. magna*) according to the Rotoxkit F™ Standard Operational Procedure (Creasel, 1990a). The test organisms were prepared by allowing cysts to hydrate in dilution water for 18–22 h prior to test initiation. Bioassays were

started by placing newly hatched rotifers in different pore water dilutions (0, 6.3, 12.5, 25, 50 and 100% v/v). Six replicates with five organisms each were used for each pore water concentration and control. Living rotifers were counted after 24 h in all control and pore water dilution replicates. The end points used for this bioassay were the 24-h LC_{50} value and the NOEC. The results were classified as follows:

- : no effect, LC_{50} and $\text{NOEC} \geq 100\%$ pore water;
- \pm : moderate effect, NOEC or $\text{LC}_{50} < 100\%$ pore water;
- + : strong effect, $\text{LC}_{50} < 50\%$ pore water.

The bioassay with the freshwater crustacean *T. platyurus* (Thamnotoxkit F™) was performed with sediment pore water (preparation as for bioassays with *D. magna*) according to the Thamnotoxkit F™ Standard Operational Procedure (Creasel, 1990b, 1992). Test organisms were prepared by allowing cysts to hydrate in dilution water for 18–22 h prior to test initiation. Bioassays were initiated by placing newly hatched test organisms in different pore water dilutions (as for Rototoxkit F™). Three replicates with 10 organisms each were used for each pore water concentration and control. Living animals were counted after 24 h in all control and pore water dilution replicates. The end point used for this bioassay was the 24-h LC_{50} value and the NOEC. The results were classified using the same criteria as for the Rotoxkit F™ bioassay.

2.5. Bioaccumulation bioassays

The accumulation bioassays with aquatic worms were performed according to the method described by Maas et al. (1993). A sediment–water system was prepared by mixing 1 l of sediment with four volumes of artificial fresh water (DSW) in a glass aquarium after which the sediment was allowed to settle for 2 days. The test was initiated by transferring 20 g of worms to the sediment–water system and exposing the organisms for 28 days at 20°C in darkness. Oligochaetes were obtained from commercially cultured stocks. The dominant species were *Limnodrilus*

claparedeianus (family: Tubificidae) and two species of the family of Naididae: *Pristina idrensis* and *Homochaeta naidina*. Before starting the bioassays the worms were kept on clean reference sediment for 1 month. During the test, oxygen concentration, pH, NO_2^- concentration, NH_4^+ concentration and conductivity were measured routinely to check whether validity criteria were met (Maas et al., 1993). The worms were fed minimally by adding 1 ml of 10% Trouvit once per week. The bioassay was terminated by washing the worms on a 250- μm mesh sieve in tap water, after which the worms were placed for 24 h in water from the sediment–water test system to allow clearance of gut contents. The worms were then transferred to a 200- μm mesh sieve in DSW. Adherent water was removed by drying the sieve for 1 min on filter paper, after which the sieve with the worms was weighed. For analysis of metals and organic contaminants, worms were transferred to polythene and glass vials, respectively, and stored at -20°C . Bioassays were performed in duplicate; for contaminant analysis the worms from two bioassays were pooled. At the start of the bioassays, unexposed worms (kept on reference sediment) were stored for chemical analysis.

Contaminant analysis in biota (and in sediment from accumulation bioassays) included heavy metals (Hg, Cd, Pb, Cu, Zn, Cr and Ni), polycyclic aromatic hydrocarbons (PAHs, 16 standard compounds proposed by US-EPA), standard polycyclic biphenyls (PCBs) (IUPAC congeners nos. 28, 52, 101, 118, 138, 153 and 180) and the following chlorinated pesticides or related transformation products: pentachlorobenzene (QCB), hexachlorobenzene (HCB), octachlorostyrene, hexachlorocyclohexane stereoisomers: α -HCH, β -HCH, γ -HCH (lindane), dieldrin, endrin, heptachlor, heptachlorepoxyde, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT and *p,p'*-DDT. Trace metals were analysed in microwave acid-digested samples with atomic absorption spectrometry (flame-, HGA- and cold vapour-AAS) according to methods previously described in Van Hattum et al. (1991, 1996a). After clean up (deactivated alumina adsorption column) PAHs were analysed in acetone/hexane Soxhlet extracts, using high performance liquid chromatography (HPLC) with fluorescence detection, as described in detail in Wegener et al. (1992) and Van Hattum et al. (1998). The PCBs and chlorinated pesticides were determined in

different fractions (deactivated silica column) of purified (deactivated alumina column) acetone/hexane Soxhlet extracts with gas chromatography with electron capture detection (GC-ECD; see Leonards et al., 1993). Quality control monitoring of the chemical analysis included analysis of procedural blanks, internal control samples and certified reference materials.

2.6. Triad approach for assessing sediment quality (situation before remediation)

The results of the different surveys, bioassays and chemical analyses were interpreted according to the Triad approach as described earlier (Den Besten et al., 1995). Site means for the abundances of taxonomic groups, mentum deformities of *Chironomus* larvae and the CCP index were classified by comparison with existing criteria, resulting in the score no effect (–), moderate effect (\pm) or strong effect (+). The criteria were based on the outcome of research in clean and moderately polluted reference sites (De Poorter et al., 1996; classification criteria published previously in Den Besten et al., 1995; Den Besten, 1997). The criteria used in the present study were those specified for stable silty sediment in shallow waters (sediment with grain size fraction $<63\ \mu\text{m}$ of 10% or more and a water content $<50\%$ w/w). For the total abundance of nematodes, only one criterion was available: the value of 10,000 animals m^{-2} was used to distinguish between strong effects ($<10,000\ \text{m}^{-2}$) and no effects ($\geq 10,000\ \text{m}^{-2}$).

Contaminant levels in biota were evaluated by comparison with Maximum Tolerable Risk levels (MTRs, also named “Maximum Permissible Concentrations or MPCs) for food-chain poisoning of birds. These MTRs represent the exposure level at which 95% of the bird species are protected (‘protected’ meaning that exposure is below the NOEC for a certain contaminant in a certain species). The MTRs were defined for specific food types (Den Besten et al., 1995; Den Besten, 1997). Moderate risk (\pm) is concluded when one or more MTRs are exceeded by a factor between 1 and 10; high risk (+) is concluded when the exceedance factor is >10 .

The Triad evaluation of sediment toxicity was based only on the results of the bioassays with *Chironomus riparius*, *D. magna* and *V. fischeri* (for

Table 1

Results of Triad approach for sediment quality assessment (results are described in more detail in Den Besten et al. (1995), Kok et al. (1995) and Van Hattum et al. (1996b); the Triad assessment was based on monitoring in 10 sampling points for each site). Numbers given are the criteria for strong effects as described in Den Besten et al. (1995) and Den Besten (1997); see also Section 2.6. Toxic units of heavy metals, pesticides and PAHs were calculated for *D. magna* by calculating for each contaminant the ratio between the normalized level in standard sediment and the NOEC (no observed effect concentration) reported in literature for *D. magna*; these ratios were totalled for the groups of heavy metals, organochlorine pesticides and PAHs. PCBs were not included because the sensitivity of *D. magna* for toxicity of PCBs is low; instead, the ratio of the level of CB-153 in sediment and the critical value for class 2 sediment quality was calculated. The critical value for PCB-153 in sediment is a target value aimed to protect against risks via accumulation in food chains (Ministry of Transport, Public Works and Water Management, 1994)

Site	Field observations indicating strong effects	Result of bioassays	Causal relationships based on calculations of toxic units from the contaminant levels in sediment	Conclusion of Triad study and prognosis of improvement after remediation
Nieuwe Merwede (1992–1995)	Low abundance of chironomids ($<100 \text{ n/m}^2$), bivalves ($<200 \text{ n/m}^2$) and nematodes ($<10,000 \text{ n/m}^2$) Levels of cadmium, mercury and PCBs in bivalves and water plants $>\text{MTR}$ or $>10 \times \text{MTR}$	Strong effects observed in the bioassay with <i>Daphnia magna</i> ; moderate toxicity for <i>Chironomus riparius</i> and <i>Vibrio fischeri</i>	Mean $\sum\text{-TU}_{\text{heavy metals}} = 6.3$ Mean $\sum\text{-TU}_{\text{pesticides}} = 0.4$ Mean $\sum\text{-TU}_{\text{PAHs}} = 5.4$ Levels of PCB-153 (used as model compound) exceed target values more than 40 times	High ecological risk caused by sediment pollution; risks will be decreased after remediation; limited re-contamination expected (improved quality of river Rhine water)
Spijkerboor (1993–1994)	Low abundance of chironomids ($<100 \text{ n/m}^2$) and nematodes ($<10,000 \text{ n/m}^2$) Levels of cadmium, mercury and PCBs in bivalves $>\text{MTR}$ or $>10 \times \text{MTR}$	Strong effects observed in the bioassay with <i>Daphnia magna</i> ; moderate toxicity for <i>Chironomus riparius</i> and <i>Vibrio fischeri</i>	Mean $\sum\text{-TU}_{\text{heavy metals}} = 3.3$ Mean $\sum\text{-TU}_{\text{pesticides}} = 0.06$ Mean $\sum\text{-TU}_{\text{PAHs}} = 2.9$ Levels of PCB-153 (used as model compound) exceed target values more than 10 times	High ecological risk caused by sediment pollution; risks will be decreased after remediation but increase again due to re-contamination (poor quality of river Meuse water)

classification of effects, see Section 2.5). The results of the chemical analyses of sediment samples were evaluated by comparison of normalized levels of contaminants with literature data on toxicity of the various compounds in each of the three bioassays (see Den Besten et al., 1995; Den Besten, 1997). Toxic units (TU) were calculated as the ratio between the level of a contaminant and the lowest reported NOEC for this compound. Sums of toxic units were

calculated for the following groups of contaminants (as a worst-case approach additivity is assumed within these groups): heavy metals, organochlorine pesticides and PAHs. Sum values ($\sum\text{-TU}_{\text{group}}$) of individual sampling points were averaged to site means. When a $\sum\text{-TU}_{\text{group}}$ exceeds 1, it is taken as an indication (\pm) for cause–effect relationships based on the (limited) group of routinely analysed contaminants. $\sum\text{-TU}_{\text{group}}$ that exceed the value of 3 are considered as stronger

Table 2

Classification of sediment contamination and contaminant levels expressed as toxic units before and after pilot-remediations in Nieuwe Merwede and Spijkerboor. Number of sampling points: Nieuwe Merwede before remediation $n = 10$; after remediation $n = 4$; non-remediated site $n = 3$; Spijkerboor before remediation $n = 10$; after remediation $n = 7$; non-remediated site $n = 3$. Toxic units were calculated as described in Table 1. Presented are mean values \pm SD

Parameter	Area	Situation studied				
		Before remediation (1993/1995)	Seven months after remediation (1996)	One year after remediation (1997)	Two years after remediation (1998)	Non-remediated reference site (1998)
Sediment contamination class (range)	Nieuwe Merwede	3–4	0–2	2–3	2–3	4
	Spijkerboor	2–4	0–2	2–3	2–3	2–4
TU _{heavy metals}	Nieuwe Merwede	6.3 \pm 1.7	1.6 \pm 0.8	1.6 \pm 0.2	1.5 \pm 0.5	3.2 \pm 0.4
	Spijkerboor	3.3 \pm 1.2	1.4 \pm 0.5	2.0 \pm 0.3	1.5 \pm 0.2	1.7 \pm 0.9
TU _{PAHs}	Nieuwe Merwede	5.4 \pm 2.5	2.6 \pm 0.9	2.5 \pm 1.0	1.6 \pm 0.7	2.6 \pm 0.1
	Spijkerboor	2.9 \pm 0.5	1.7 \pm 0.3	2.6 \pm 0.3	1.3 \pm 0.5	1.7 \pm 0.5
TU _{organochlorine pesticides}	Nieuwe Merwede	0.38 \pm 0.43	0.05 \pm 0.004	0.05 \pm 0.01	0.05 \pm 0.04	0.14 \pm 0.06
	Spijkerboor	0.06 \pm 0.01	0.03 \pm 0.0001	0.03 \pm 0.01	0.08 \pm 0.02	0.03 \pm 0.01
Ratio of PCB-153/critical value for class 2 sediment quality	Nieuwe Merwede	41.6 \pm 19.5	5.2 \pm 1.3	4.0 \pm 0.7	3.8 \pm 2.2	21.2 \pm 10.9
	Spijkerboor	12.2 \pm 4.4	6.4 \pm 1.7	5.2 \pm 0.5	6.6 \pm 1.7	3.7 \pm 0.6

indications (+) for causal relationships (because effects can really be expected at contaminant concentrations three times or more above the NOEC level).

The final Triad results was obtained by comparing the site scores of the most sensitive parameter of 'field observations', of 'bioassays' and the highest Σ -TU_{group}. For combinations of the three categories with one or more '+', and at minimum '±' for the other categories, high ecological risk caused by sediment pollution was concluded.

2.7. Evaluating the effect of remedial action

A comparison of sediment quality before and after the pilot-remediations and in non-remediated sites (see Fig. 1) was made using the following parameters:

- *Sediment contamination*: overall classification; toxic units calculated for *D. magna* or for PCBs, the factor by which the Dutch target value for the PCB-153 level in sediment is exceeded (PCB-153 chosen as indicator for PCBs; target value aims at the protection of top predators against risks via accumulation in food-chains).

- *Effects in bioassays*: the number of tests showing moderate or strong effects (classified as \pm or +), out of a total of five different bioassays (see Section 2.4).
- *Macrofauna*: abundances, biomass, incidence of mentum deformities of *Chironomus* larvae, CCP index, species diversity of different taxonomic groups and overall diversity indices (see Section 2.3).
- *Nematodes*: total abundance, species diversity and the maturity index (Bongers, 1990).
- *Bioaccumulation*: contaminant uptake measured in the accumulation bioassay with oligochaetes, evaluated by comparing remediated and non-remediated sediment, and by comparing the contaminant levels in the oligochaetes with MTRs for foodchain poisoning (see also Section 2.6; Den Besten et al., 1995; Den Besten, 1997).

The results are presented as site means \pm SD or as ranges. The differences were tested by ANOVA and Student's *t*-test.

3. Results

The selection of the pilot remediation sites in the

Table 3
Bioassay responses before and after pilot-remediations in Nieuwe Merwede and Spijkerboor. In total five different bioassays were used. Results for October 1996 and March 1997 were similar. No data available for the non-remediated site in the Nieuwe Merwede in 1996/1997. For number of sampling points, see Table 2

Parameter	Area	Before remediation (1993/1995)	Seven months and 1 year after remediation (1996 and 1997)	Two years after remediation (1998)	Non-remediated reference site (1996 and 1997)	Non-remediated reference site (1998)
Number of bioassays showing a strong response (range)	Nieuwe Merwede	1–3	0–1	0–1	–	0–1
	Spijkerboor	0–2	0–1	0–1	0–1	0–1
Number of bioassays showing a moderate or strong response (range)	Nieuwe Merwede	1–4	1	2	–	2–3
	Spijkerboor	1–4	0–1	1–2	0–1	1–2

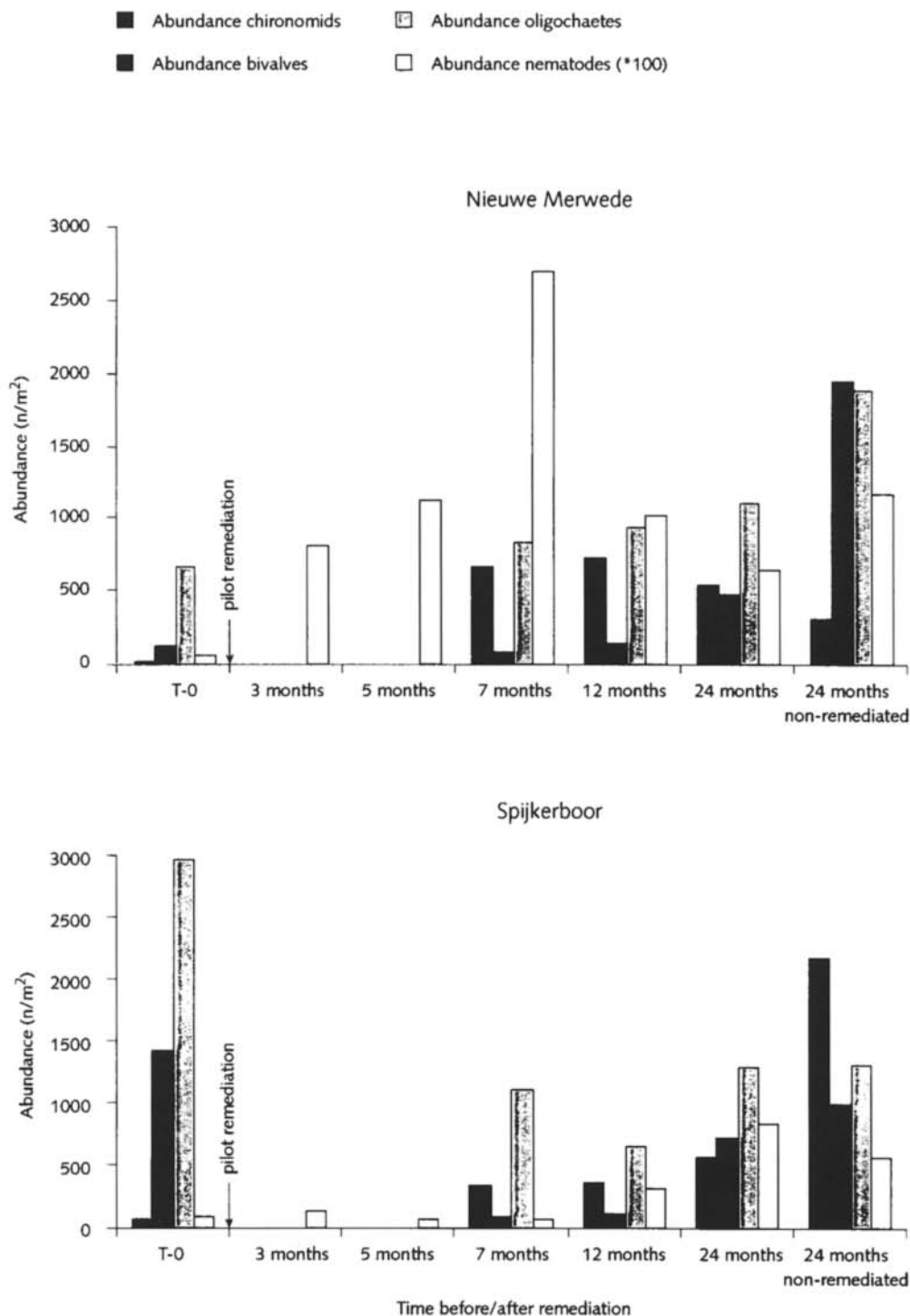


Fig. 2. Results of macro- and meiofauna surveys before and after pilot remediations in the Nieuwe Merwede and Spijkerboor. Bars represent mean values for variable numbers of sampling points (for macrofauna each sample in triplicate): Nieuwe Merwede before remediation $n = 10$; after remediation $n = 4$; non-remediated site $n = 3$; Spijkerboor before remediation $n = 10$; after remediation $n = 7$; non-remediated site $n = 3$. For the monitoring 3 and 5 months after the remediations only meiofauna (nematodes) was investigated.

Nieuwe Merwede and the Spijkerboor was based on the outcome of a sediment quality assessment according to the Triad approach, the results of which are summarized in Table 1. Based on research carried out in 1992–1995, for both sites high ecological risks were concluded. Sediment pore water caused strong effects on the water flea, *D. magna*. The bioassay with *D. magna* appeared to be more sensitive than the tests with *Chironomus riparius* and *V. fischeri* (not shown). Strong effects were also observed in the bioassays with *Thamnocephalus* and *Brachionus* (not included in Triad evaluation). The calculations of toxic units for *D. magna* indicated that the levels of heavy metals and PAHs in particular are high enough to cause effects in the bioassay with this species (Table 1).

The removal of polluted sediments and capping of the remaining deeper sediment with clean sand was carried out between autumn 1995 and spring 1996. In the first 6 months after the remediations the sediment top layer remained sandy, but after 1 year in both sites a top layer of several centimetres of silty sediment had formed. In Table 2, a comparison is made between the degree of sediment contamination (classified according to the Dutch criteria for sediment quality) before and after the pilot remediations. The contaminant levels were lowest immediately after the clean up, while 1 year later, class 2 to 3 levels were again found. However, in comparison to the class 4 levels before the remediations, and especially considering the lower number of contaminants on which the classification was based, the sediment remained less polluted. A decrease in the sediment contaminant levels of non-remediated sites was also observed. In the Spijkerboor creek in 1998 comparable values were found for the sums of toxic units in the sediment of the remediated and the non-remediated site (Table 2). By contrast, in the remediated site in the Nieuwe Merwede toxic unit values remained lower than in the non-remediated site (Table 2). The net reduction (comparing the remediated site with the non-remediated site for the year 1998) was about 50% for heavy metals, nearly 40% for PAHs and 64% for organochlorine pesticides. The net reduction in the level of PCBs was 82% (based on levels of CB-153).

Table 3 summarizes the results of the bioassay studies. In the period 1996–1998 it was found for both the remediated and the non-remediated sites

that per sampling point, only one bioassay out of five tests showed a strong effect (cf. per sample up to three bioassays gave a strong response in the experiments performed in 1993/1995). The strong bioassay response was observed either in the test with *Daphnia* or in the one with *Thamnocephalus*, both crustacean species. The bioassays with *Chironomus riparius* and *V. fischeri* showed moderate sediment toxicity in a limited number of sampling points, but no responses were observed in the bioassay with the rotifer *B. calyciflorus*. When moderate effects are included, a maximum of two bioassays showed an effect in the same sampling point from the remediated sites, while two or three bioassays responded in the non-remediated sites of the Spijkerboor and the Nieuwe Merwede (cf. up to four bioassays before the remediation; Table 3).

The changes in the abundances of the different taxonomic groups of macrofauna are shown in Fig. 2. A rapid recolonization was observed for chironomids, oligochaetes and nematodes in the remediated site in the Nieuwe Merwede (for nematodes within 3 months). After 7 and 12 months the abundances of the chironomids and nematodes were already much higher than before the remediation (cf. Table 1). However, in the non-remediated site the abundances in 1998 were higher than before the clean up (Fig. 2). The abundance of bivalves showed a continuous increase over the period 1996–1998. In the remediated site of the Spijkerboor after 7 months only oligochaetes were present at a normal abundance. A continuous increase in abundance of chironomids, bivalves and nematodes occurred from 1996 to 1998. Two years after the remediation of the site in the Spijkerboor, the abundances of chironomids and nematodes were much higher than before the remediation. However, these changes were also observed in the non-remediated site (Fig. 2).

In Table 4 a comparison is made between the situations before and after the remediations for other macrofauna parameters. Additional information about species composition is given below. The differences in the abundance of macrofauna between the remediated and non-remediated sites described above are reflected by differences in the biomass of the different macrofauna groups. Species diversity within the group of chironomids showed an increase in both the remediated and non-remediated sites, and

Table 4

Characterization of the macrofauna community before and after pilot-remediations in Nieuwe Merwede and Spijkerboor. Presented are mean values \pm SD. For number of sampling points (for macrofauna each sampling point sampled in triplicate) see Table 2. Abbreviations: CCP index — ratio between the abundance of *Chironomus* larvae and the total of *Chironomus* and *Procladius* species; AFDW — ash free dry weight; NA — not analysed (insufficient numbers of *Chironomus* larvae). *denotes significant difference in comparison to situation before remediation ($p < 0.05$)

	Nieuwe Merwede			Spijkerboor		
	Before remediation (1995)	After remediation (1998)	Non-remediated (1998)	Before remediation (1993)	After remediation (1998)	Non-remediated (1998)
Number of chironomid species	1 \pm 1	8 \pm 2*	6 \pm 1*	2 \pm 1	3 \pm 2	3 \pm 2
Biomass of chironomids (g AFDW/m ²)	0.01 \pm 0.01	0.32 \pm 0.24	0.08 \pm 0.03	0 \pm 0	0.05 \pm 0.05	0.34 \pm 0.23
CCP index	0.56 \pm 0.47	0.59 \pm 0.27	0.98 \pm 0.03	0.02 \pm 0.04	0.08 \pm 0.2	0.0 \pm 0.0
Mentum deformities of <i>Chironomus</i> species(%)	NA	22 \pm 17	64 \pm 31	NA	NA	NA
Number of oligochaete species	7 \pm 1	7 \pm 2	7 \pm 1	6 \pm 1	7 \pm 1	7 \pm 1
Biomass oligochaetes (g AFDW/m ²)	1.2 \pm 0.6	0.6 \pm 0.3	1.5 \pm 0.6	1.8 \pm 2.4	0.8 \pm 0.2	0.5 \pm 0
Number of bivalve species	9 \pm 2	8 \pm 1	11 \pm 1	8 \pm 2	9 \pm 2	10 \pm 2
Biomass bivalves (g AFDW/m ²)	3.9 \pm 2.0	2.3 \pm 0.4	10 \pm 9.5	20.4 \pm 36.2	6.6 \pm 5.7	29.4 \pm 12
Number of species Ephemeroptera, Trichoptera and Plecoptera	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	1 \pm 1	0 \pm 0
Diversity index according to Simpson	0.13 \pm 0.01	0.16 \pm 0.05	0.13 \pm 0.03	0.73 \pm 0.07	0.19 \pm 0.07*	0.27 \pm 0.09*
Diversity index according to Shannon–Wiener	2.23 \pm 0.1.0	2.27 \pm 0.15	2.42 \pm 0.13	1.95 \pm 0.37	2.16 \pm 0.28	1.93 \pm 0.25

to a larger extent in the Nieuwe Merwede than in the Spijkerboor (Table 4). Before the remediations the low abundance of chironomids in the Nieuwe Merwede and the Spijkerboor was dominated by *Procladius* sp. In the case of Nieuwe Merwede, *Chironomus acutiventris* and *Chironomus bernensis* were also found. In the remediated site of the Nieuwe Merwede, 7 months after the remediation, the latter two species and *Chironomus nudiventris* had become relatively abundant (more than 100 m^{-2}). Two years after the remediation, *Chironomus acutiventris* and *Chironomus bernensis* were still found at relatively high abundances, together with *Polypedilum bicrenatum* and *Procladius* sp. The incidence of mentum deformities determined in the survey of 1998 was higher in the non-remediated site than in the remediated site, but the difference is not statistically significant ($p > 0.05$; Table 4). In the Spijkerboor especially *Einfeldia carbonaria* had reappeared 7 months after the remediation. Two years later the chironomids were still dominated by this species, while in addition low abundances of *Cryptochironomus* sp. with *Polypedilum bicrenatum* and *Procladius* sp. were found. The absence of *Chironomus* species in Spijkerboor is also seen by the low CCP index (Table 4).

For oligochaetes, the situation in the Nieuwe Merwede and the Spijkerboor before and after remediation showed no differences with regard to the number of species (Table 4) and the species composition. In all cases, the oligochaetes were dominated by *Limnodrilus hoffmeisteri*, *Limnodrilus claparedeianus*, *Potamothrix moldaviensis* and Tubificidae without hair cetae. Also for bivalves, the number of species 2 years after remediation was comparable with the situation before remediation (Table 4). Seven months after the remediations in the Nieuwe Merwede and the Spijkerboor, different *Pisidium* species were found at very low densities (*Pisidium casernatum/casernatum* f. *plicata*, *Pisidium moitesierianum*, *Pisidium supinum*). Two years after the remediations the species composition was comparable to that in the non-remediated sites, although a few species were absent (Table 4).

Results of calculations of more general indices for species diversity (including all taxonomic groups investigated, e.g. gastropods), such as the Simpson and the Shannon–Wiener indices, indicate minor

differences between the remediated and non-remediated situation in the Nieuwe Merwede. For Spijkerboor a somewhat higher mean value of the Shannon–Wiener index was found for the remediated site, whereas the Simpson index was lower (both changes indicating a higher diversity). In addition, the Simpson index was considerably lower in both the remediated and non-remediated site when compared to the situation in 1993 (differences significant at $p < 0.05$; Table 4). Interestingly, the remediated site in the Spijkerboor was the only site where in 1998 species of the Ephemeroptera (mayflies) were found.

The results of the nematode surveys are as follows. Three months after the remediations in the Nieuwe Merwede and the Spijkerboor (June 1996), respectively, 22 and 15 nematode taxa were found (mean values; cf. 17 and 25 before the remediation). In 1998, in the remediated site of the Nieuwe Merwede on average 24 taxa were found, which was higher than in the non-remediated site (11 taxa) and also higher than before the clean up. In comparison to the non-remediated site more nematode species were found that are regarded as sensitive to disturbance, and a more complete trophic structure was present, with omnivorous, carnivorous and fungivorous species being present. Species belonging to the family of Tripylidae were present in all samples from the remediated site, but not in samples from the non-remediated site. This shift in species composition is also reflected in the maturity index, which tended to be higher in the remediated site (in 1998: ranging 2.5–2.8, compared to 2.1–2.6 for the non-remediated site). In the Spijkerboor, in 1998, comparable numbers of nematode taxa were found in the remediated and the non-remediated site (20–21 taxa). However, in the Spijkerboor marked differences in species composition were observed. After the remediation the relative abundance of the nematodes belonging to the family Halaphanolaimidae (genera *Aphanolaimus* and *Paraphanolaimus*) decreased from more than 20% to values below 5%, while in the non-remediated site it remained higher than 20%. Within this group, the genus *Paraphanolaimus* reacted strongly (from 15% before to <1% after the remediation, in October 1996 and April 1997, followed by an increase to 3% in April 1998). The maturity index, however, showed no differences between the remediated and the non-remediated site in the Spijkerboor: in 1998 the

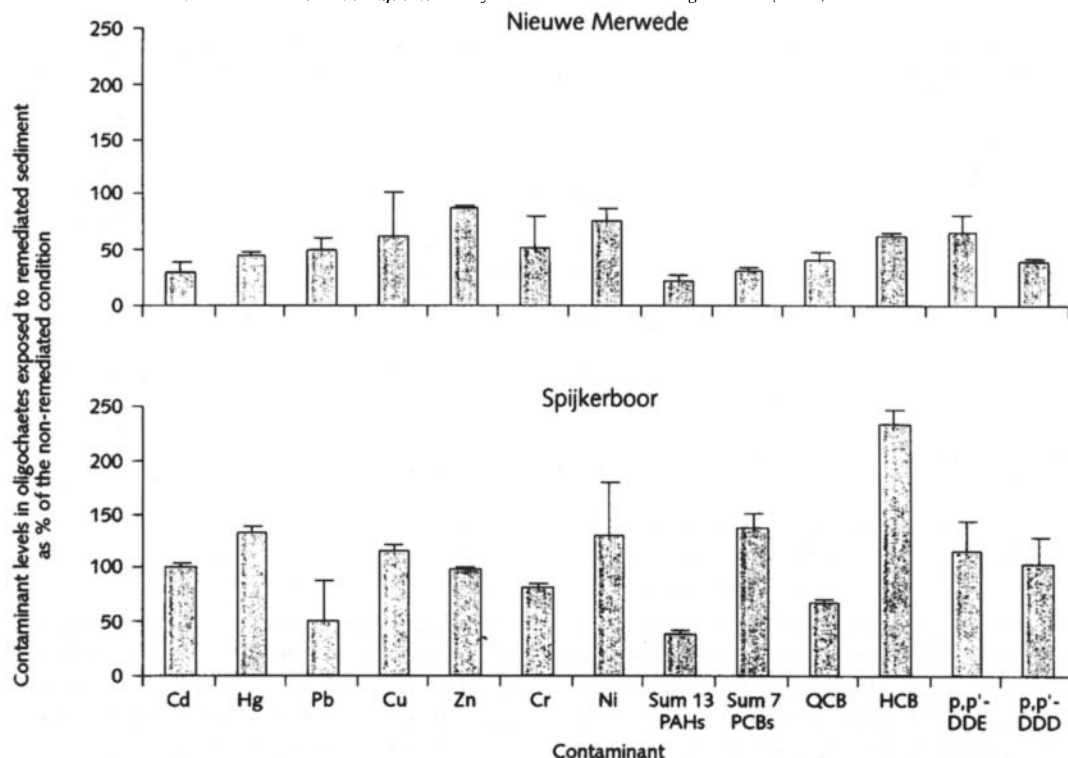


Fig. 3. Results of 28-day accumulation bioassays with oligochaetes. Bars represent levels in oligochaetes from remediated sediment as percent of levels in oligochaetes from non-remediated sediment (mean % \pm SD). For each site, sediment from three sampling points was tested. The sum PAHs consists of 13 individual PAHs that were measurable above detection limit; organochlorine pesticides listed in Methods but not shown here were below detection limit.

observed ranges for the two sites were 2.3–2.8 and 2.5–2.7, respectively.

In September 1997 (18 months after the pilot remediations), bioaccumulation in oligochaetes in sediment from the remediated sites was compared with contaminant uptake from non-remediated sediment. In sediment from the remediated site in the Nieuwe Merwede, bioaccumulation was lower compared to the non-remediated situation (Fig. 3 and Table 5). However, the levels of Cd, Hg and PCB-153 (as an indicator for the accumulation and risks of the group of toxic, planar PCBs) still exceeded maximum tolerable risk levels for food chain poisoning (Table 5).

4. Discussion

The pilot remediation projects in the Nieuwe

Merwede and the Spijkerboor were set up for various purposes, part of them being technical, logistical and so on, in preparation for a possible large-scale clean up operation in the delta of the rivers Rhine and Meuse. It was realized at the start of the projects that it would be extremely difficult to remove all contaminated sediment from a site. In the case of the project in the Nieuwe Merwede there was also concern about the possibility of increased infiltration of contaminated water after the removal of thick layers of silt (Van Meel et al., 1997). For these reasons it was decided to remove only part of the contaminated sediment and to cap the remaining contamination with clean sand. With regard to the risks for the ecosystem such an approach can be successful when enough clean material is supplied, ensuring that there will be no mixing of old and fresh layers of sediment as a result of erosion processes or bioturbation.

Direct influence of the remedial actions was related

Table 5

Risk assesment based on bioaccumulation bioassays with sediment from pilot remediations in Nieuwe Merwede and Spijkerboor. Only contaminants are shown which (nearly) exceed MTR criteria. Mean MTR-exceedance values are based on chemical analyses performed on material from three sampling points (September 1997)

	MTR ^a (µg/kg w/w)	Level in oligochaetes/MTR				
		Nieuwe Merwede, remediated sediment	Nieuwe Merwede, non-remediated sediment	Spijkerboor, remediated sediment	Spijkerboor, non-remediated sediment	Reference sediment (oligochaete culture)
Cd	6	20.8	72.8	48.9	53.4	7.3
Hg ^b	13	5.9	13.7	5.1	4.3	2.0
PCB-153 ^c	4	4.5	8.6	4.3	3.0	0.5
HCB	69	0.05	0.05	0.06	0.03	<0.01
<i>p,p'</i> -DDE	21	0.11	0.12	0.05	0.05	0.03
<i>p,p'</i> -DDD	15	0.14	0.25	0.08	0.08	0

^a MTR = maximum tolerable risk level for risk of food chain poisoning of birds. MTRs are based on literature data of toxicity experiments with birds; final MTRs were corrected for food type (assimilation efficiency) and energy content (Den Besten et al., 1995; Den Besten 1997).

^b Risk assessment for mercury is based on the assumption that accumulated levels are present as methyl-Hg.

^c PCB-153 is used as guiding compound for the more toxic planar PCBs (De Boer et al., 1993; Den Besten, 1997).

to the resuspension of contaminated sediment in the water and subsequent water flow to areas close by. This led to for example increased accumulation levels in freshwater mussels (Pieters, 1995). This effect may have been stronger in the Spijkerboor than in the Nieuwe Merwede, because of the higher current velocities in the latter watercourse.

The (net) positive effect of the remedial actions will clearly depend on the quality of the suspended solids carried by the river that will form the new top layer of the sediment, thereby recontaminating remediated sites. Especially for the Nieuwe Merwede, a limited recontamination was expected after the remediation. However, the results of the monitoring programme after the clean-up show that in both sites, within 1 year the levels of some contaminants in the sediment had increased to class 3 levels again, although they remained lower than before the clean-up. These findings are in agreement with levels of contaminants measured routinely in suspended solids from the rivers Rhine and Meuse which vary between classes 1 and 3 (Den Besten, 1997; Van Eck et al., 1997). This means that not all contaminants in newly formed sediment can meet the quality criteria for minimal protection of all species theoretically present (when class 1 quality is achieved, a theoretical 95% of the species is protected based on single compound evaluations as described by Aldenberg and Slob, 1991). The net effect of remediation in the Spijkerboor may be

lower than in the Nieuwe Merwede because erosion by wind and as a result of shipping activities may result in a stronger exchange of sediment between the remediated and the non-remediated site than is the case in the Nieuwe Merwede.

In addition, the net positive effect of the pilot remediations in the Spijkerboor and the Nieuwe Merwede is lower than expected as a result of the natural improvement of sediment quality in the non-remediated sites. The influence of 'natural' improvement in the non-remediated site of the Nieuwe Merwede is not expected to be as strong as in the non-remediated site of the Spijkerboor because erosion/sedimentation rates in the groyne sections of the Nieuwe Merwede are low (sedimentation rates were high in the 1970s until an equilibrium between sedimentation and erosion was reached, see Den Besten et al., 1995). In addition, bioturbation may result in a mixing of the top layer with deeper layers, which in the case of the Nieuwe Merwede are highly polluted.

For the evaluation of the improvement of sediment quality, a selection was made of parameters from the sediment quality Triad. In the case of bioassay responses, the sediment toxicity was evaluated by a larger number of tests than in the standard Triad approach. The effects observed in the bioassays were presented as the number of tests (or species) giving a moderate or strong response. It can be argued that this approach can be related better to the method

by which the Dutch criteria for contaminant levels are derived (e.g. the criteria that need to be met for class 1 quality are based on the aim to protect 95% of the species in the aquatic environment, see above).

Although the decrease of sediment contamination from class 4 to class 3 indicates only a limited improvement, the observations made with the battery of bioassays indicate a decrease in the number of bioassays showing effects in the newly formed sediment. This is in accordance with earlier observations indicating that the quality of suspended solids of the rivers Rhine and Meuse has improved considerably over the last decades, resulting in a decreased toxicity of samples from surface waters (see Den Besten et al., 1995). Taking together bioassays that showed moderate or strong effects, a tendency of increasing toxicity in time after the remediations was observed (from 0–1 to 1–2 bioassays showing response). The pilot remediation in the Nieuwe Merwede resulted in a slightly better sediment quality in comparison to the non-remediated situation (in the latter 2–3 bioassays gave a response). The bioassay with *D. magna* appeared to be the most sensitive test; indeed the calculations of toxic units indicate that the levels of metals and PAHs may not have decreased sufficiently to rule out effects on this species. At the same time, for continuation of the monitoring programme it seems worthwhile to include more sensitive bioassays in the battery.

The observed changes in the abundance (and biomass) of chironomid larvae, oligochaetes and nematodes suggest a more rapid recolonization in the Nieuwe Merwede compared to the Spijkerboor. Stream velocities in the Nieuwe Merwede (part of the river Rhine) are higher than in the Spijkerboor. Besides reproduction, drift has been reported to be an important mechanism for colonization (Mackay, 1992). The rate of recovery of the benthic community clearly depends on the invertebrate mobility. Bivalves, being sedentary organisms, showed the slowest recolonization in the present study. Dramatic changes of the macrofauna species composition within 3 months were also reported by Dudok van Heel et al. (1993) who carried out a translocation experiment with containers filled with sediment from different areas of the Rhine–Meuse delta. In the latter study these changes were governed by simultaneous changes in sediment characteristics,

because the sediments were placed in not exactly comparable stream conditions. By contrast, in the present study colonization by macro- and meiofauna was studied during/after the renewal of a silty top layer with sediment characteristics comparable to the situation before remediation.

The observations on the macrofauna showed that of all parameters studied, especially the abundances and species diversity of the different taxonomic groups responded to the improvement of sediment quality in the remediated and non-remediated sites. The incidence of mentum deformities could also be a parameter that is sensitive enough, but in the present study insufficient numbers of *Chironomus* larvae could be investigated to show significant differences. As well, the number of rare insect species (like mayflies) may provide a sensitive index. Indices like the Simpson index, the Shannon–Weaver index or, in the case of the nematodes, the maturity index showed only minor changes. Nevertheless, most of these changes also indicate an improvement of sediment quality. From the nematode surveys it became clear that the change in the relative abundance of specific families or genera could be useful to indicate changes in sediment quality.

Bioaccumulation in sediment from the remediated site in the Nieuwe Merwede was lower than in sediment from the non-remediated sites. For most contaminants this was not the case for Spijkerboor. This can be explained by the fact that the contaminant levels in the remediated and non-remediated sediment of the Spijkerboor are comparable, whereas this is not the case in the Nieuwe Merwede. However, in all sites investigated, the levels of Cd, Hg and PCB-153 (as an indicator for the accumulation of the group of toxic PCBs, see De Boer et al., 1993; Den Besten, 1997) still exceed maximum tolerable risk levels for the risk of food-chain poisoning. This is consistent with the fact that, although after the remediation the PCB-153 levels in the Nieuwe Merwede sediment have dropped by a factor of more than 10 (and a net reduction of 82% compared to non-remediated sediment), the PCB-153 levels in new sediment still do not meet the environmental quality objectives (score = class 2; minimal protection is realized when class 1 levels are achieved; the long-term objective is to have class 0 sediment).

The results of the biological monitoring in polluted,

non-remediated sites indicate a comparable improvement of the quality of the sediment top layer as in the pilot remediation sites of Nieuwe Merwede and Spijkerboor. An exception seems to be the accumulation of contaminants in biota in the Nieuwe Merwede, which was considerably lower in the remediated site compared to the non-remediated site. The improved macrofauna and decreased sediment toxicity in the non-remediated sites are likely to be the result of natural sedimentation of material with lower contaminant levels (see comments above). Therefore for the long-term development of the Rhine–Meuse delta it can be expected for some sites that the risks for the ecosystem will decrease without remedial actions. It is concluded that the priority for remedial action has to be based not only on the outcome of risk assessment, but also on predictions of the rate by which polluted sediment is covered naturally with cleaner sediment from the rivers Rhine and Meuse.

Acknowledgements

This work was financed by the Directorate of Zuid-Holland, Rijkswaterstaat, The Ministry of Transport, Public works and Water management. The authors would like to thank all persons involved in the extensive monitoring programme. In particular, Ad Schipperen (Directorate of Zuid-Holland), Loek Knijff and EricJan Houwing (RIZA) are acknowledged for their part in planning and performing the sampling trips. Albert van Espeldoorn (RIZA), Lia Kerkum and other personnel of AquaSense Laboratory and BLGG are acknowledged for their work on the bioassays and the nematode and macrofauna analyses. Personnel of Alcontrol (Raamsdonkveer, Netherlands) and the Institute for Environmental Studies (Vrije Universiteit Amsterdam) are greatly acknowledged for their work on contaminant analysis. Finally, thanks are due to Thomas Arts (Directorate of Zuid-Holland), Bram bij de Vaate (RIZA) and Jan Hendriks (RIZA) for their valuable comments on the manuscript.

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